Available online at www.sciencedirect.com

SCIENCE DIRECT®

Developmental Biology 269 (2004) 1–17

DEVELOPMENTAL BIOLOGY

www.elsevier.com/locate/ydbio

Review

A hidden program in *Drosophila* peripheral neurogenesis revealed: fundamental principles underlying sensory organ diversity

Eric C. Lai[a,1] and Virginie Orgogozo[b,*,1]

[a] *Howard Hughes Medical Institute, 545 Life Sciences Addition, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3200, USA*

[b] *UMR 8542, École Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France*

Received for publication 25 September 2003, revised 22 January 2004, accepted 26 January 2004

Abstract

How is cell fate diversity reliably achieved during development? Insect sensory organs have been a favorable model system for investigating this question for over 100 years. They are constructed using defined cell lineages that generate a maximum of cell diversity with a minimum number of cell divisions, and display tremendous variety in their morphologies, constituent cell types, and functions. An unexpected realization of the past 5 years is that very diverse sensory organs in *Drosophila* are produced by astonishingly similar cell lineages, and that their diversity can be largely attributed to only a small repertoire of developmental processes. These include changes in terminal cell differentiation, cell death, cell proliferation, cell recruitment, cell–cell interactions, and asymmetric segregation of cell fate determinants during mitosis. We propose that most *Drosophila* sensory organs are built from an archetypal lineage, and we speculate about how this stereotyped pattern of cell divisions may have been built during evolution.

Keywords: Peripheral nervous system; Cell lineage; *Drosophila*; Insect; Evolution; Asymmetric cell division; Apoptosis; Cell recruitment; Notch; Numb; EGF

Introduction

Essential pursuits of developmental biology include understanding how cells communicate with each other, how cells are committed to survive, die, or proliferate, and how undifferentiated cells adopt terminal cell fates. These processes are key to the formation of biological patterns and thus underlie the organized development of multicellular life. The extreme morphological diversity and stereotyped development of insect external sensory organs have made them an ideal setting for studying these issues, and studies of insect sensilla have now been pursued for over a century (Berlese, 1909).

First studies concentrated on understanding the cell complement of multicellular adult peripheral sensory organs. A varying number of cells were found, with one or more sense cells bearing specialized sensory dendrites associated with one to three modified epidermal cells. Although peripheral sensory organs were generally admitted to originate from the epidermis, an early longstanding controversy concerned the nature of the sense cell. From 1909 to 1941, some authors claimed that the sense cell is prolonged by the axon of a centrally located neuron (Berlese, 1909; Franzl, 1941; Haffer, 1921; Vogel, 1923). However, other studies from this time and into the 1950s convincingly demonstrated that the same cell of an individual peripheral sensillum extends a dendrite to the periphery and projects an axon to the central nervous system, indicating that the "sense" cell and the neuron are indeed one and the same (reviewed by Bate, 1978; Bullock and Horridge, 1965).

Continuing studies of insect sensilla and certain non-sensory structures (primarily non-innervated butterfly scales) led to the general understanding that cells of an individual sensillum are typically derived from divisions of a common progenitor cell (reviewed by Bate, 1978; Lawrence, 1966; Peters, 1963). However, no consensus emerged with regard to numbers, orientations of cell divisions, and fates of the different cells in different sensory organs and species. Thus, it seemed possible that various

* Corresponding author. Present address: Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544. Fax: +1-609-258-1712.

E-mail address: virginie.orgogozo@normalesup.org (V. Orgogozo).

[1] These authors contributed equally to this work.

0012-1606/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2004.01.032

peripheral sensilla may have evolved independently of each other.

From multiple revisions during the last 5 years, a new picture of the microchaete bristle lineage in *Drosophila melanogaster* has recently emerged. Surprisingly then, its comparison with other studies and a re-examination of previously unconnected observations now reveal an astonishing concord among cell lineages that produce very morphologically distinct sensory organs. This strongly suggests that diverse sensory organs evolved from the same ancestral lineage. We investigate here the probable characteristics of this ancestral sensory lineage and propose models for the evolution of sensory lineages in *Drosophila* based upon limited modifications to the canonical lineage.

The history of the thoracic microchaete lineage

The small mechanosensory bristles that decorate the thorax of the adult fruitfly, referred to as microchaetes (Fig. 1A), have been a premier model system for investigating the mechanisms of cell fate determination. Their accessibility, stereotyped spatial pattern, and relatively synchronous development make their study comparatively convenient. A large number of molecular markers now permit the unambiguous identification of the different cells in the sensory lineage. Together with biochemical analyses of the relevant proteins and increasingly sophisticated in vivo genetic studies, a detailed picture of the molecular and cellular events underlying bristle development has emerged.

The ontogeny of these mechanoreceptors is conceptually similar to that of most peripheral sensilla (reviewed by Jan and Jan, 1993). Within a field of otherwise undifferentiated epithelial cells, groups of adjacent cells termed proneural clusters acquire neural potential due to the spatially patterned expression and activity of proneural proteins, which are basic helix–loop–helix (bHLH) transcriptional activators. There are two subtypes of proneural protein, the Ato class (Atonal and Amos) and the AS-C class (Achaete, Scute, and Lethal of Scute); external mechanoreceptors are specified from clusters of Achaete- and Scute-expressing cells. Neural potential is subsequently restricted to a single cell in each cluster, the single sensory organ precursor (SOP), in a process mediated by the Notch (N) signaling pathway. Once stably determined, the SOP undergoes a series of asymmetric cell divisions that are also regulated by the N pathway, giving rise to the different cells comprising an individual microchaete organ.

Despite intense research on microchaete development throughout the 1990s, fundamental descriptions of its very lineage and cell composition have undergone multiple revisions in recent years. We first consider the history of microchaete lineage analysis and trace how available techniques and thinking about the lineage have evolved over the years.

In the beginning there were four: first models of the microchaete lineage

The most easily observed features of microchaete sensilla are elaborated by two cells that produce a socket and the bristle shaft (Fig. 1B) (Lees and Waddington, 1942; Robertson, 1936). Accordingly, early genetic studies focused on mutations that affected their development (Lees and Waddington, 1942). However, additional cells of the organ lie entirely beneath the cuticle, including the neuron itself (Fig. 1C) (Stern, 1938). Detailed studies of these cells were for many years limited by the lack of available means to specifically visualize them during development.

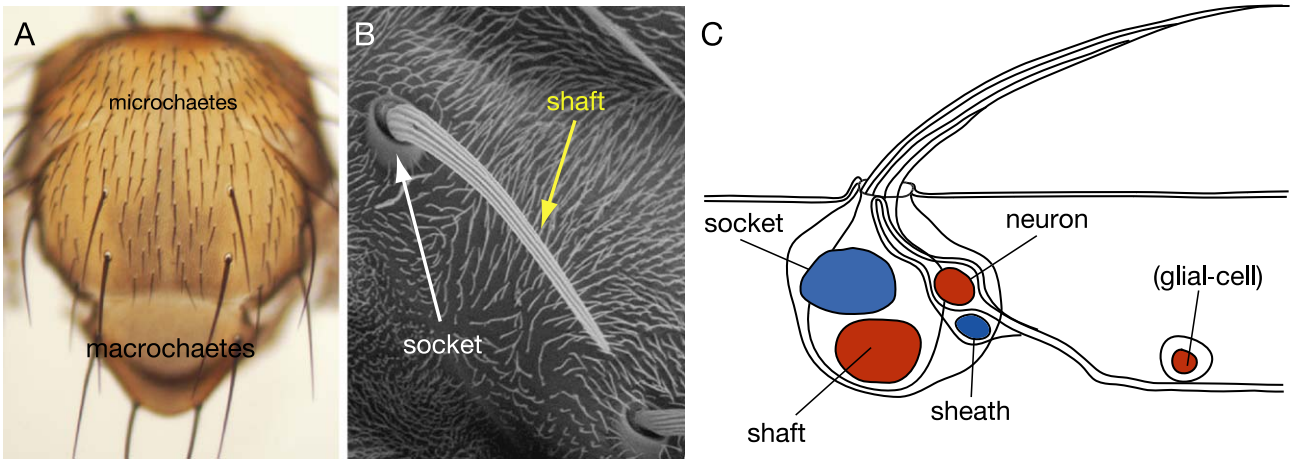


Fig. 1. *Drosophila* adult mechanoreceptors. (A) Preparation of the dorsal thorax of an adult fruitfly showing the ordered array of mechanosensory bristles. The smaller bristles are referred to as microchaetes while the larger bristles are known as macrochaetes. (B) Scanning electron micrograph showing the external structures produced by two cells in the mechanosensory organ, the socket and the shaft. (C) Schematic cross section of a mechanosensory organ; the socket and shaft cells are endoreplicated and larger than the remaining internal cells of the sensillum. Notch signaling is activated during specification of the socket and sheath cells (blue nuclei) and is inactive during specification of remaining cells (red nuclei). The glial cell is designated in parentheses because it has recently been found to undergo apoptosis (see Fig. 2 and text for details).

The first detailed descriptions of the cellular composition and lineage of adult *Drosophila* sensilla were undertaken by Hartenstein and Posakony (1989). Their electron microscopy analysis indicated that microchaetes are composed of one neuron and three support cells, the socket cell, the shaft cell, and a sheath cell which enwraps the neuron (Fig. 1C). Their studies also took advantage of the observation that many support cells in the sensory lineage react with the "neuronal" marker MAb 22C10, which facilitated their identification during development. They also exploited the fact that cells in the microchaete sensory lineages are the only ones to divide and/or undergo endoreplication in the epithelium of the pupal notum. This allowed mitotic cells in the sensory lineage to be specifically labelled with BrdU, a technique also used to analyze sensory lineages in the embryo (Bodmer et al., 1989). They concluded that the four bristle cells derive from two successive rounds of divisions of an SOP (also referred to as pI; Fig. 2A). A fifth cell was observed to incorporate BrdU during the lineage divisions and seen to associate with the neuronal axon, but its clonal origin could not be established by these methods. It was proposed at this time to be a glial cell of some sort and to be potentially homologous to the "soma sheath cell" of larval sensilla (Fig. 2A) (Hartenstein, 1988). However, based on the distance between it and the other daughters of the SOP, it was hypothesized that it might arise from the adepithelial cell layer, which lies basally to the epithelium proper.

Later studies were made possible by the *lacZ* enhancer trap A101, an insertion at the *neuralized* locus (Boulianne et al., 1991). In this genetic background, the SOP and its daughters can be specifically identified by their accumulation of β-galactosidase (Huang et al., 1991). In agreement with the previous study, they observed that successive divisions of macrochaete SOPs generated four cells, but that a "fifth" A101-positive cell could be identified at many developing sense organ positions. A fifth cell was similarly noted in some developing microchaete sensory organ clusters (Usui and Kimura, 1993). However, this cell was proposed to be recruited to the developing sensory organ based on its apparently de novo expression of *lacZ* at a distance from the daughters of the SOP (Huang et al., 1991).

On the basis of these studies, a model for the microchaete lineage became generally accepted in which the SOP undergoes three asymmetric divisions to generate four cells (Fig. 2A) (reviewed by Posakony, 1994). Division of the SOP, was proposed to generate two daughters, one of which (pIIa) gives rise to the two large endoreplicated cells that produce exterior structures [the socket cell (tormogen) and shaft (trichogen)] and one (pIIb) that gives rise to two subepidermal cells [the sheath cell (thecogen) and the neuron]. The existence of such a "four-cell" sensory lineage was bolstered by genetic experiments in which cell divisions in the sensory lineage could be made symmetric by altering the activity of various components in the N pathway. For each pair of sister cells in this description of the SOP lineage, one cell is a net N signal-sender, while the other is a net N signal-receiver.

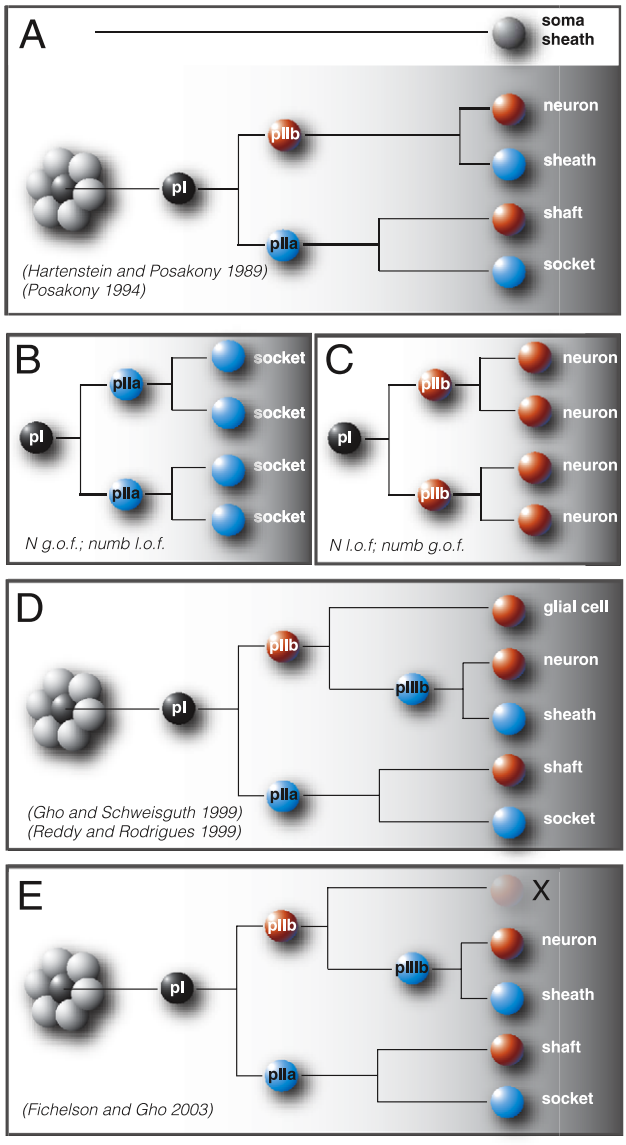


Fig. 2. Evolving models of the microchaete lineage. Following its selection from a proneural cluster (darkened cell in the cluster), the sensory organ precursor (pI) cell executes a fixed lineage to generate the cells of the mechanosensory organ. As in Fig. 1, cells that activate the N pathway are blue, whereas cells that do not (typically due to inheritance of Numb) are red. (A) In the original models proposed by Hartenstein and Posakony, pIIa divides first to generate the socket and shaft cells, then pIIb divides to generate the sheath cell and neuron. A fifth cell (the soma sheath cell) was noted to be associated with developing sense organs but not thought to be clonally related. (B, C) Manipulation of N pathway activity alters the fate of cells in the lineage. In *N* gain-of-function (g.o.f.) or *numb* loss of function (l.o.f.) experimental conditions, four-socket sensory organs can be observed (B), while *N* loss-of-function or *numb* gain-of-function conditions can result in four-neuron organs (C). (D) Subsequent model by the Schweisguth and Rodrigues labs showed that pIIb divides first to generate a glial cell and pIIIb. pIIa then divides to generate the socket and shaft cells, then pIIIb divides to generate the sheath cell and neuron. Note that pIIIb is the same cell as pIIb of panel A. (E) Revised model by the Gho lab showing that the glial cell undergoes apoptosis (X), leaving four cells in the mature sensillum.

Excess N signaling (due to misexpression of N pathway components or loss of the N antagonist Numb) causes both daughters to adopt the fate of a signal-receiving cell, while the absence of N signaling (due to mutations in the N pathway or Numb misexpression) causes both to adopt the fate of a signal-sending cell. The extreme outcomes of such perturbations are four socket (Fig. 2B) and four neuron (Fig. 2C) sensory organs, respectively (Hartenstein and Posakony, 1990; Parks and Muskavitch, 1993; Rhyu et al., 1994; Schweisguth et al., 1996). This model was consistent with the attractive hypothesis that the N pathway was successively exploited over the course of sensory organ evolution to generate non-neuronal support cells from a theoretical "neuron-only" progenitor sensory lineage.

And then there were five: the glial cell joins the lineage

In the following years, the molecular mechanisms of sensory cell fate determination were pursued more aggressively. As the sequence of events in the cell lineage became defined at higher resolution, a discrepancy in the accepted model emerged about the relative timing of the cell division producing the neuron and sheath cell. It was originally shown to occur *after* pIIa cell division (Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989). However, subsequent studies that used the homeodomain transcription factor Prospero (Pros) as a cell marker definitively showed that the sister cell of pIIa divides *before* pIIa (Manning and Doe, 1999; Reddy and Rodrigues, 1999b).

Several explanations to resolve these conflicting data were put forth, including the existence of an additional, previously unrecognized cell division in the sensory lineage (Manning and Doe, 1999). This was indeed shown to be the case (Gho et al., 1999; Reddy and Rodrigues, 1999a). Notably, the study of Gho and colleagues developed the powerful technique of live imaging of sensory development using sensory organ-specific expression of GFP and time-lapse confocal microscopy. This strategy allowed divisions within the SOP lineage to be followed real-time, and clearly showed that four, and not three, divisions occur. Additional experiments using fixed material clearly substantiated this view and led to a new lineage for microchaetes. In particular, the pIIa sister cell (pIIb) does divide before pIIa, but one of its daughters (now referred to as pIIIb) subsequently undergoes an additional cell division to then generate the sheath cell and neuron (Fig. 2D). It is worth reiterating that due to this change in nomenclature, the cell that produces the neuron and sheath cell, referred to as pIIb in reports before mid-1999 (Gho and Schweisguth, 1998; Hartenstein and Posakony, 1989), is presently referred to as pIIIb.

The revised lineage nicely resolved issues regarding the division order and the mysterious origin of the frequently observed "fifth" cell. The fifth cell was further established to be a small glial cell, as it expresses glial-specific markers such as Glial cells missing (Gcm) and Repo (Gho et al., 1999; Reddy and Rodrigues, 1999a). All other glial cells (with the exception of the midline glia) are similarly derived from neural lineages (Jones, 2001), thus making the origin of this mysterious cell analogous to most other types of glia. Many classical studies, including some focusing on cockroach and cricket sensilla, similarly described a fifth, possibly glial, basal cell that is associated with the neuron and sheath cell (Gnatzy, 1976; Gnatzy and Schmidt, 1971; Lawrence, 1966). This cell is likely to correspond to the fifth glial cell of *Drosophila* microchaetes. Interestingly, live imaging indicated that the glial cell of *Drosophila* microchaetes migrates subepidermally away from the remaining four cells of the lineage (Gho et al., 1999), which remain closely apposed. The previous lack of molecular markers specific for this small cell, combined with its migration, thus seemed to adequately explain how its existence had been previously overlooked.

Back to four: apoptosis of the glial cell

Although the migratory nature of the glial cell seemed sufficient to explain its absence from the mature mechanosensory organ, it was noted that it might in principle undergo apoptosis (Reddy and Rodrigues, 1999a). Indeed, the latest analysis of the microchaete lineage showed that the glial cell undergoes programmed cell death shortly after its birth (Fig. 2E) (Fichelson and Gho, 2003). This observation was facilitated by a highly localized and strongly fluorescent marker (histone 2B::YFP) that permitted higher resolution live imaging of nuclear events, including fragmentation of the glial cell nucleus. Genetic experiments provided evidence that the glial cell dies by programmed cell death, since glial nuclear fragmentation is suppressed in *H99* mutant clones, in which the pro-apoptotic genes *grim*, *reaper*, and *head involution defective* are absent, or in cells expressing the viral caspase inhibitor p35. Finally, glial cell fragments were shown to be phagocytosed by macrophages. As these cells are mobile, it was proposed that the previous report in which the glial cell was found to be migratory involved analyses of unusually large glial cell nuclear fragments that were engulfed by macrophages, which subsequently travelled some distance.

Does the short-lived glial cell have a function with regard to microchaete development, or does its death symbolize that it is an evolutionary relic or vestige? Glial cells in other developmental settings are well known to have functions in axonal pathfinding and neuronal survival. However, in the microchaete lineage, the glial cell dies before growth cones begin to be extended, and in some cases fragmentation is observed even before division of pIIIb (Fichelson and Gho, 2003). This suggests that the glial cells are not likely to strongly influence the normal development of the microchaete neuron. When apoptosis is blocked, surviving glial cells are indeed associated with axons for some time and axonogenesis occurs slightly prematurely. This is perhaps indicative of an ancestral function of glia during sensory organ formation. Nevertheless, this situation has no major

developmental or behavioral consequences, so the rationale for glial apoptosis is mysterious at present.

Although each successive version of the microchaete lineage had in its time come to be generally accepted as correct, there is reason to believe that current analyses have been sufficiently detailed to now truly reflect "the truth". This hope is supported by comparisons with other peripheral sensory lineages, which collectively reveal an ancestral lineage that underlies the development of a dizzying array of insect sensilla.

Variations on a theme—principles underlying diversification of related sensory organ lineages

The thoracic microchaetes are but one of many types of peripheral sense organ present in embryonic, larval, or adult *D. melanogaster*. These include other mechanosensory organs and chemosensory (olfactory and gustatory) organs, all of which are external sensory organs, as well as chordotonal (proprioceptive and auditory) organs, which are internal sensory organs (Fig. 3). Within each class, sensory organs show additional morphological diversity. For example, mechanosensory organs can appear as long bristles (macrochaetes), small bristles (microchaetes), bristles of intermediate sizes, thorn bristles, slender bristles, domes (campaniform sensilla), bifurcated hairs, and so forth. Each multicellular sensory organ is innervated by one or more neurons that bear a ciliated sensory dendrite (Fig. 3A); these have been collectively termed type I neurons (Zawarzin, 1912). In addition, there exist multidendritic (md) neurons devoid of accessory cells (Fig. 3B) that are present internally in the embryo, larva, and adult (Bodmer and Jan, 1987; Jan and Jan, 1993). These neurons are unciliated and classified as type II neurons; their sensory modalities are largely unknown.

Until 1999, it was thought that these various organs, all containing different numbers and types of cells, were produced from distinct classes of cell lineages. However, recent studies suggest a common canonical lineage program underlying the formation of these diverse sensory organs.

The canonical lineage

On the basis of the evidence discussed below, we propose that the canonical lineage consists of four successive cell divisions (Fig. 4A). The first division of the sensory organ precursor (pI) occurs within the plane of the epithelium and generates an epithelial-like cell (pIIa) and a neuroblast-like cell (pIIb). Then, the pIIa cell divides and produces two outer cells. Closely following pIIa mitosis, pIIb divides perpendicularly to the plane of the epithelium and generates an inner cell that may migrate away, as well as a precursor (pIIIb) of a sensory neurone and its closely associated cell. The microchaete lineage (Fig. 2E) is an obvious variation of this canonical lineage: the inner cells form the socket and shaft cells, the migrating cell is a dying glial cell and the neuron-associated cell form the sheath cell. We now discuss the evidence in support of this canonical peripheral sensory lineage and show how a limited set of modifications to this core lineage, including changes in terminal cell fate, lineage proliferation, lineage apoptosis, and cell recruitment, may provide sensory organ diversity.

Changing sense organ types: the gap between internal and external sensory organs is cut

Generation of distinct sensory organs via similar lineage strategies requires differential activation of gene expression batteries appropriate for sensory organ subtype. The easiest way to accommodate this is through the selective expression of different transcription factors in different lineages. The overall choice between external and internal sensory organ illustrates this principle well.

Chordotonal organs are internalised stretch-sensitive sense organs linked to the cuticle. These organs are often made up of a complex cluster of closely associated sensory structures individually known as scolopidia (Moulins, 1976), and each scolopidium derives from an independent pI cell (Bodmer et al., 1989; Brewster and Bodmer, 1995; Okabe and Okano, 1997). At least some scolopidia are composed of five cells: a scolopale cell that enwraps the neuronal dendrite, and three other cells (attachment, cap, and ligament cells) that link either extremity of the sensory organ to the cuticle (Fig. 3D) (Brewster and Bodmer, 1995; Ghysen and O'Kane, 1989; Hartenstein, 1988; Matthews et al., 1990). Although more than one model has been proposed for the scolopidial lineage (Bodmer et al., 1989; Brewster and Bodmer, 1995), one of them has retrospectively been noticed to be highly analogous to the revised microchaete and md-es lineages (Fichelson and Gho, 2003; Orgogozo et al., 2001), suggesting a one-to-one correspondence between the cells in these morphologically dissimilar organs (Figs. 4B, C, E).

According to this view, the cap and attachment cells correspond to the socket and shaft cells, while the scolopale cell corresponds to the sheath cell. Our examination of the literature produces strong molecular support for this hypothesis. First, the cap and attachment cells express the socket and shaft enhancer-trap marker A1-2-29 (Blochlinger et al., 1991; Hartenstein and Jan, 1992), while the scolopale cell expresses the sheath cell marker Prospero (Doe et al., 1991; Vaessin et al., 1991). Furthermore, the ligament cell, which corresponds to the glial cell, accumulates the glial markers Gcm, Repo, and Wrapper (Campbell et al., 1994; Halter et al., 1995; Jones et al., 1995; Noordermeer et al., 1998; Xiong et al., 1994). Eventually, the aligned arrangement of the scolopidium cells is comparable to the arrangement of the different cells of certain stretched external sensory organs (Figs. 3D, E).

The lineal relation between chordotonal and external sensory organs is further reinforced by genetic studies of

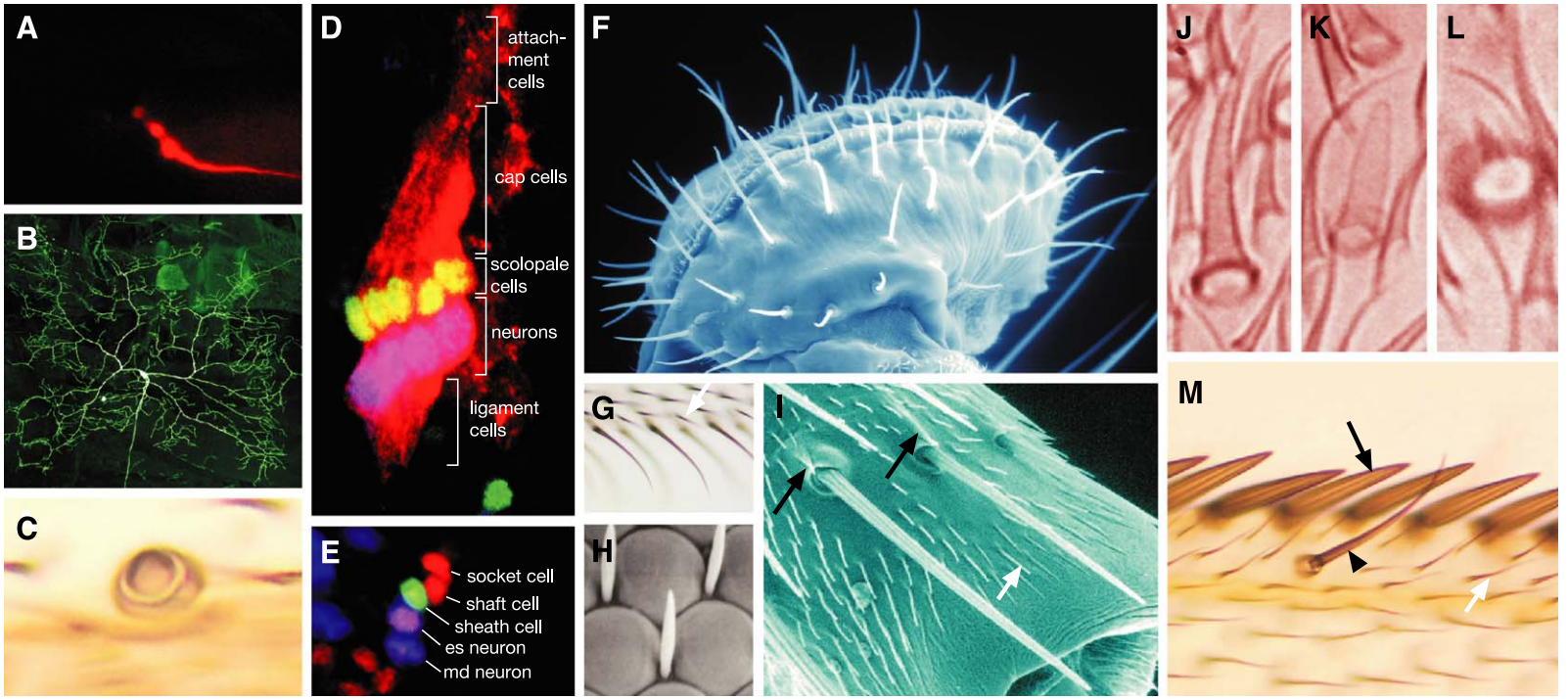


Fig. 3. Morphologically diverse sensory organs in *Drosophila*. (A) Typical bipolar neuron of an adult external mechanosensory bristle organ, as visualized by MAb 22C10/α-Futsch. (B) Multidendritic neuron (v'ada) as visualized by expression of mCD8-GFP. (C) Campaniform sensillum located on the L3 wing vein. (D) Embryonic lch5 chordotonal organ showing expression of β-galactosidase in red (to detect expression of *atonal(5.1)-lacZ*), Prospero in green, and Elav in blue; anterior is left and dorsal is up. The lch5 organ comprises five scolopidia arranged in parallel. (E) A single lh2 external sensory organ from the embryonic abdominal region, labelled for Cut in red, Prospero in green, and Elav in blue. Dorsal is left, posterior is up. (F) Labellum of the adult proboscis showing rows of taste bristles. (G) Posterior wing margin showing non-innervated modified mechanoreceptors. The neighboring cuticular structures are not sensory and are named trichomes (red nuclei). (H) Close view of the adult eye showing interommatidial bristles. (I) Leg mechanosensory bristles are associated with non-lineally related bract cells (black arrows); epidermal cell produce non-sensory trichomes (white arrow). (J–L) Three types of olfactory sensilla: trichoid (J), basiconic (K), and coeloconic (L). (M) Anterior wing margin showing stout mechanosensory bristles (arrow) and a slender mechanosensory bristle (arrowhead); non-sensory trichomes produced by wing cells (white arrow).

Cut. Cut is a homeodomain-containing protein that accumulates in all external sensory cells and their precursor cells but not in internal sensory cells (Blochlinger et al., 1990). Loss of *cut* activity transforms external sensory organs into chordotonal organs (Bodmer et al., 1987; Merritt, 1997; Merritt et al., 1993), while ectopic Cut expression results in the reciprocal transformation (Blochlinger et al., 1991). Thus, Cut has an instructive role in execution of an external-type peripheral sense organ program. The switch can be further linked to the different proneural proteins that initiate development of these sensory organs: Achaete and Scute proneural proteins direct the development of external mechanoreceptors, which express Cut in pI and all of its daughters, while Atonal directs the development of all chordotonal organs at least in part by repressing Cut (Jarman and Ahmed, 1998). Thus, individual pI cells have certain multipotent properties, and the specific type of sensory organ program they execute is influenced by expression of selector genes.

Changing terminal lineage fates: Gcm redirects a presumptive neuron to the glial fate

Multidendritic (md) neurons (lacking accessory cells) are found at many stages throughout *Drosophila* life (Fig. 3B). A subset of these are positioned near external sensory organs (Ghysen et al., 1986), and retrospective studies indicated that individual SOP cells indeed give rise to both md neurons and campaniform external sensory (es) organs (Brewster and Bodmer, 1995; Vervoort et al., 1997). In the larva, md neurons exhibit specific dendritic arborizations within characteristic regions of the epithelium, suggesting that they are receptive to stimuli different from the one received by campaniform organs (Grueber et al., 2002). Thus, these embryonic pI cells produce two functionally independent sensory organs, one campaniform organ and one md neuron.

The nature of the lineage that produces these md and es organs was controversial for some time, and the md neuron has been variously proposed to be the sibling of the es neuron (Brewster and Bodmer, 1995) or born of a hypothetical "p0" cell and thus sibling to pI (Vervoort et al., 1997). Careful observation of the successive cell divisions demonstrated that the md neuron is actually born from pIIb and is sibling to pIIIb (Orgogozo et al., 2001). Therefore, the md-es lineage is identical to that of the microchaete lineage except that an md neuron is produced in place of the apoptotic glial cell of the latter (Figs. 4B, E). The likeness of the microchaete glial cell and md neuron is supported by the observation that both cells migrate some distance away from their initial position, whereas their sibling sensory organ cells stay closely apposed. The overall relationship between these lineages is also reflected by the common order of cell divisions: pIIb first, pIIa second, and pIIIb last.

Certain wing campaniform sensilla have similarly been observed to produce two neurons, one of which extends a typical external sensory dendrite and one that displays characteristics of an md neuron (Murray et al., 1984). Although it was proposed that the md-like cell is the sibling of the sensory neuron (Van De Bor et al., 2000), in light of recent data on the embryonic campaniform lineage, we suggest that it too is born of pIIb, and sibling to the cell that subsequently produces the external sensory neuron and sheath cell.

Because the md neuron/glial cell is the only cell in the lineage that never activates the N pathway (Gho et al., 1999; Van De Bor et al., 2000), it is in some sense the basal cell type in the sensory lineage (Figs. 4A, B, E). The fact that this cell adopts a glial fate in some lineages is therefore at odds with the popular conception that neurons represent the basal state of sensory organs. Interestingly, in *gcm* mutants, the glial cell produced by the microchaete lineage and the gliogenic campaniform lineage (see below) is transformed into an Elav-positive neuron (Fichelson and Gho, 2003; Van De Bor et al., 2000), indicating that the glial cell in both lineages retains some neural potential. Taken together, these data suggest that embryonic campaniform (Fig. 4B) and md neuron-associated wing campaniform organ lineages (not shown) are more representative of the canonical lineage, while the microchaete (Fig. 4E) and gliogenic campaniform lineages (Fig. 4G) are more derived and have selectively acquired expression of Gcm.

A remarkably similar set of principles may apply to the development of chordotonal organs. Firstly, some embryonic chordotonal organs that lack the glial-like ligament cell have been specifically shown to have lineage relation to an md neuron (Brewster and Bodmer, 1995). According to BrdU incorporation studies (Bodmer et al., 1989) and in light of the new data, we propose that both the md neuron and the chordotonal organ cells arise from a mixed chordotonal-md lineage with the same pattern of cell divisions as the mixed campaniform-md lineage. Secondly, in *gcm* mutants, the ligament cells of the embryonic lch5 chordotonal organ are transformed into neurons and each scolopidium becomes bi-innervated by two Elav-positive neurons (Jones et al., 1995), perhaps revealing an ancestral bi-innervated sensory organ. Indeed, the *D. melanogaster* auditory organ located on the antenna consists of an array of ~100 scolopidia, each of which lacks a ligament cell but instead contains two sensory neurons whose dendrites insert into the same scolopale (Eberl et al., 2000; Hertweck, 1931).

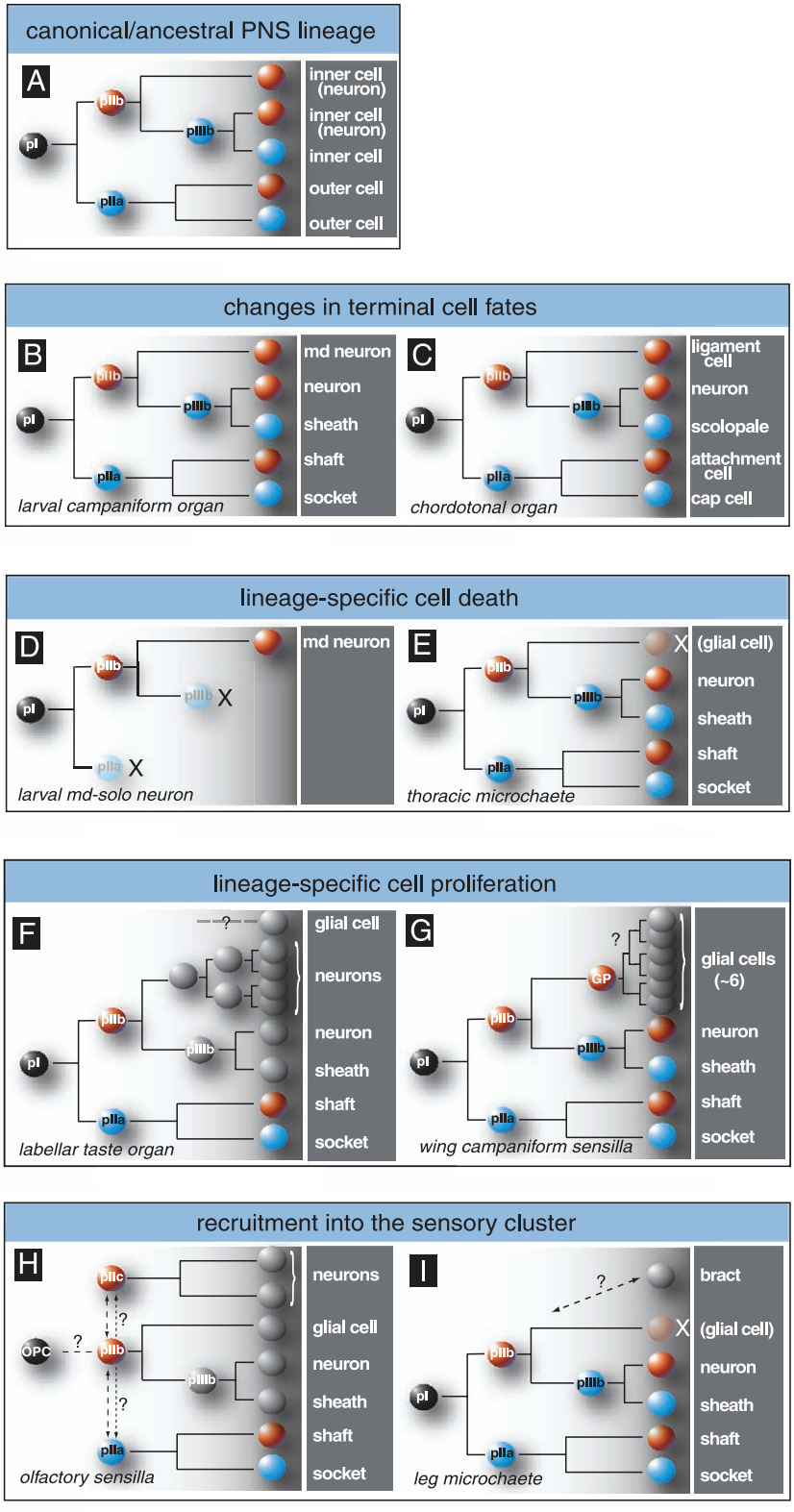
In summary, a neuron-basal state can be revealed in multiple extant lineages, whose diversification into glial/ligament cells is attributable to Gcm function.

Lineage-specific cell proliferation

Cell proliferation can add to a sensory organ's complement. Analysis of the development of some wing campaniform organs (Fig. 3C) showed that their primary precursor cell produces the four sensory organ cells and a Gcm- and Repo-positive glial cell through the exact same stereotyped pattern of cell divisions as in the microchaete lineage (Van

De Bor and Giangrande, 2001). However, the glial cell does not undergo apoptosis in this case but proliferates to produce an average of six glial cells that migrate away (Fig. 4G). The molecular control of the surprisingly variable fates of glial cells—to survive, die, or proliferate—remains to be fully understood, but is clearly dependent on cellular context.

Additional cell divisions probably also occur for sensory organs composed of more than four sensory cells. For example, leg and labellar taste bristles typically comprise one mechanosensory and four chemosensory neurons in addition to the three accessory cells (Nayak and Singh, 1983; Ray et al., 1993). Accordingly, BrdU incorporation



studies indicated that the lineage of the five-neuron labellar chemosensory bristles (Fig. 3F) is highly similar to the canonical lineage, except that the cell that corresponds to the md neuron/glial cell undergoes additional cell divisions to produce four chemosensory neurons (Ray et al., 1993). However, this lineage model does not account for the formation of the glial cell detected next to chemosensory neurons in flies and other insects (Lawrence, 1966; Nayak and Singh, 1983; Peters, 1963), suggesting that an additional cell division may occur within this lineage.

All *Drosophila* taste organs appear to originate from Cut-positive precursor cells that specifically accumulate the transcription factor Pox-neuro (Poxn) (Dambly-Chaudiere et al., 1992). In *poxn* mutants, poly-innervated taste organs are transformed into mono-innervated sensory organs, whereas ectopic expression of *poxn* leads to the opposite transformation (Awasaki and Kimura, 1997, 2001; Dambly-Chaudiere et al., 1992; Nottebohm et al., 1994a). Interestingly, *poxn* activity is independent of *cut*. Expression of *poxn* in sensory precursors is maintained in *cut* mutants, with resultant transformation of external poly-innervated organs into internal, but still poly-innervated organs (Vervoort et al., 1995). Thus, *poxn* regulates the number of cell divisions within lineages. There is variability in neuronal numbers amongst taste bristles, with distinct subclasses being innervated by two, three or four neurons (Ghysen et al., 1986; Nayak and Singh, 1983). This suggests that additional factors may act in concert with Poxn to regulate neuron number appropriately in different taste bristle lineages.

Apoptosis shapes sensory organs

Many examples of cell death in developing metazoan nervous systems have been described. However, unlike the glial death prescribed by fate seen in the microchaete lineage, other cases usually involve death through stochastic mechanisms. For example, many midline glial cells die during *Drosophila* embryogenesis as a result of glial cell competition for Spitz, an EGF receptor ligand secreted by neurons and required for glial survival (Bergmann et al., 2002; Sonnenfeld and Jacobs, 1995; Zhou et al., 1997). An analogous mechanism underlies the massive neuronal death observed during vertebrate CNS development. Here, neurons compete for limiting amounts of neurotrophic factors produced by target cells, with survival of only a small fraction of neurons that establish successful connections (Raff et al., 1993). Excess uncommitted cells are also directed to programmed cell death during fly eye development (Wolff and Ready, 1991).

In contrast, detailed studies of the complete lineage of *Caenorhabditis elegans* demonstrate that the death of specific, pre-determined cells is an essential feature of nematode development (Sulston and Horvitz, 1977; Sulston et al., 1983). Although few examples of lineage-fated cell death have been described outside of nematodes, this developmental tactic may prove to be used more frequently than is currently appreciated. Indeed, examination of the literature produces additional possible examples of cell deaths that have been programmed into neural lineages in *Drosophila*. For example, the posterior wing margin is lined with non-innervated mechanoreceptors that consist only of shaft and socket cells (Fig. 3G). Although their lineage has not been studied in detail, it is reasonable to suppose that they derive from a modified bristle lineage in which pIIb undergoes programmed cell death. Similar observations have been made with non-innervated wing scales of Lepidoptera, where one of the pI daughter cells undergoes stereotyped cell death (Galant et al., 1998; Stossberg, 1938). Conversely, studies of interommatidial bristles in the *Drosophila* eye (Fig. 3H) have suggested that outer cells of these modified mechanoreceptors die, leaving behind only the neuron and possibly the sheath cell (Cagan and Ready, 1989; Perry, 1968). Thoracic macrochaete shaft cells of different flies have also been found to die following completion of morphogenesis [*Calliphora vicinia*, (Keil, 1978; Ribbert, 1967) and *D. melanogaster*, T. Keil, personal communication], an outcome suggested to be related to their highly polyploid status.

The best-studied case of neural lineage death aside from the microchaete occurs during development of some embryonic md-solo neurons. Unlike md neurons produced from md-es lineages, md-solo neurons are not associated with external sense organs. Although it might be reasonably supposed that md-solo neurons arise de novo, some were

Fig. 4. Proposed relationships between diverse PNS lineages and an ancestral canonical lineage. As in Fig. 2, cells that activate the N pathway are colored blue, those that do not (typically through inheritance of Numb) are colored red. Cells derived from divisions whose control by the N pathway is not yet established are colored grey. (A) The canonical PNS lineage is similar to that shown in Fig. 2B. The larval multidendritic neuron-campaniform mechanosensory (md-mes) organ (B) and chordotonal organ (C) both follow the canonical lineage and differ only in their adoption of terminal cell fates. (D) The larval multidendritic (md) neuron lineage is similar to that of the larval md/mes organ, except that pIIa and pIIIb undergo apoptosis (X). The lineages of the adult microchaete (E) and gliogenic wing campaniform sensilla (G) also follow the canonical lineage except that the glial cell undergoes apoptosis in the former but proliferates in the latter (via a glial precursor, GP). The number and order of the GP divisions is not known in detail (question mark). (F) Proposed lineage for the taste bristles of the labellum. Multiple rounds of cell divisions produce additional neurons. A glial cell is associated with these sensory organs but its clonal relation to the sensory cluster has not yet been established (question mark). (H) Lineage of the coeloconic olfactory sensillum. An olfactory precursor cell (OPC) recruits additional cells into a three-cell presensillum cluster of a pIIa-like, pIIb-like and pIIc cell. The olfactory precursor cell is here proposed to be pIIb, but this has not been experimentally established (question mark). As well, the order of cell recruitment (double arrows) into the presensillum cluster is not yet known (question mark). The pIIa-like and pIIb-like cells execute sublineages characteristic of the canonical lineage. The number of neurons in an olfactory sensillum appears to be slightly variable (not shown). Note that in non-coeloconic olfactory lineages, the glial cell undergoes apoptosis. (I) A subset of microchaetes (on the leg and proximal costa) recruits a bract cell (double arrow). The microchaete-generating portion of this lineage is presumed to be the same as for thoracic microchaetes, but this has not been directly demonstrated.

recently found to derive from an md-es-type lineage in which both pIIa and pIIIb cells specifically activate the pro-apoptotic genes *reaper* and *grim* and undergo programmed cell death (Fig. 4D) (Orgogozo et al., 2002). When apoptosis is blocked, both of these cells divide to produce socket/shaft and a neuron/sheath cell pairs, respectively, resulting in an apparently normally formed ectopic external sensory organ. Thus, to produce a single multidendritic neuron, the canonical lineage program is launched and undesired cells are removed by apoptosis.

In the case of the md-solo lineage, the Notch-inhibitory protein Numb is inherited by the surviving daughter cell of each cell division, suggesting that execution is positively regulated by N activity (Fig. 4D). Accordingly, Numb is necessary and sufficient to prevent apoptosis, while activated Notch is able to ectopically trigger death in this lineage (Orgogozo et al., 2002). By contrast, it is the glial cell of the microchaete lineage that dies (Fig. 4E), the only cell in this lineage that never activates the N pathway (Fichelson and Gho, 2003). When considered alongside the deaths in the lineages of non-innervated mechanoreceptors and interommatidial bristles, it is clear that there is no consistent linkage between N activation and induction of apoptosis.

Depending on cell context then, Notch may induce (md-solo lineage), inhibit (microchaete lineage) or have no influence on apoptosis (e.g., pI cell division in most lineages). Cell death is triggered by pro-apoptotic genes in the *H99* region during both md-solo and microchaete development (Fichelson and Gho, 2003; Orgogozo et al., 2002; White et al., 1994), and it will be a challenge to understand how this genomic region integrates N signaling with other cues and signals to selectively activate pro-apoptotic genes in the appropriate cells of the relevant lineages. A final point is that microchaete glial death is independent of its glial identity: in *gcm* clones, the extra neuron obtained at the expense of the glial cell fate still dies (Fichelson and Gho, 2003). This suggests that glial cell apoptosis is not a simple consequence of adoption of this fate.

Joining the club: EGFR signaling recruits sensory cells

Non-lineally related cells may also be recruited into some sense organs. This is the principal mechanism for assembly of the *Drosophila* eye following selection of founder R8 cells, and recruitment relies upon reiterative use of EGFR signaling (Freeman, 1996). Relatively few other peripheral sense organs conscript cells, but those that do so also utilize EGFR signals as a general strategy to recruit cells into a developing sensillum.

Cell recruitment is used during the development of most poly-scolopidial chordotonal organs. In the embryo, the pentascolopidial organ lch5 (Fig. 3D) arises from five pI cells, each of which executes its characteristic lineage (see above). Initially, three pI cells classically arise from an *atonal*-positive proneural cluster. Then, the most ventral pI cell provides a source of Spitz/EGFR signalling which is necessary for the other two pI cells to appear (Okabe and Okano, 1997; zur Lage et al., 1997). This highlights two mechanisms for creation of a poly-scolopidial organ: multiple pI cells may be specified simultaneously from a proneural cluster, or pI cells may be recruited sequentially by an earlier specified pI cell. Both processes are used during lch5 development. In a similar manner, the adult femoral chordotonal organ arises from a group of some 70–80 pI cells in the leg imaginal disc during third larval instar and early pupa (zur Lage and Jarman, 1999). SOPs are progressively recruited from a persistent proneural cluster and accumulate in a large SOP mass. The newly formed chordotonal SOPs are the source of Spitz signalling that allows recruitment of new SOPs. Finally, the dorsalmost lch5 SOP does not recruit other chordotonal SOPs, but instead uses Spitz/EGFR signaling to recruit an average of six non-neural oenocytes (Elstob et al., 2001; Rusten et al., 2001). Overall, there is tremendous variability in the number of recruited elements in different settings: one (vch chordotonal SOP), two (lch5 chordotonal SOP), four to nine (oenocytes), and potentially many more (femoral chordotonal organ SOPs). Experimental stimulation of EGFR signaling increases the number of cells recruited in each of these settings, indicating that the level of EGFR signaling must be carefully controlled to give the desired outcome.

Neurogenesis of chordotonal organs and R8 photoreceptors is initiated by the Ato-type proneural protein, Atonal. This has led to speculation that Ato-type proneural proteins may be disposed to activate programs of gene expression that lead to clustering or recruitment of cell types, potentially by directly activating one of more components of the EGF receptor pathway (zur Lage et al., 1997). However, cell recruitment is not entirely restricted to sensory organs determined by Ato-type proneural proteins. Unique mechanosensory bristles located on the legs and the proximal costa of the wing, specified by AS-C class proneural proteins, are associated with a small cuticular protrusion secreted by a specialized epidermal cell, the bract (Fig. 3I). Clonal analysis indicated that the bract cell is not lineally related to the cells of the bristle organ but requires a neighboring bristle organ for its specification (Held, 2002 and references therein). Recent studies now demonstrate that the bristle cells induce the bract fate in an adjacent cell through Spitz/EGFR signalling via the Ras/MAPK pathway (del Alamo et al., 2002; Held, 2002). As is the case for chordotonal organ induction, the number of bract cells recruited can be experimentally modulated by manipulating the level of Spitz/EGFR signalling. The sensitive period for bract induction occurs at the three- or four-cell stage of the bristle lineage (Nottebohm et al., 1994b) and genetic and pharmacological studies suggest that the socket and/or shaft cell may be responsible for bract induction (Held, 2002 and references therein). The physiological function of the bract is not known.

Like poly-scolopidial chordotonal organs, some external sensory organs in the *Drosophila* larval anterior region are

composed of multiple neurons and support cells that seem to represent an aggregation of several sensory units. For example, the three large organs of the antenno-maxillary sensory complex comprise around 10 clustered sensilla units, while the Keilin organ possess five type I neurons innervating a cluster of three bristles (Campos-Ortega and Hartenstein, 1985). Spitz/EGFR signaling is required for the development of some of the neurons and cuticular structures of these organs, and enhancement of EGFR signaling results in greater numbers of these sensillar components (Mayer and Nusslein, 1988; Okano et al., 1992). Thus, formation of poly-unit external sensory organs also involves EGFR-mediated cell recruitment.

Common features of sensory lineages and the evolutionary origin of the canonical lineage

The collected observations indicate a preponderance of similarities amongst diverse types of peripheral sensory lineages in *Drosophila*. We cannot rule out that these similarities are the end-result of complicated evolutionary routes that led to a convergence of developmental schemes. However, the parsimony principle suggests that the most likely explanation is that most, if not all, peripheral sensory lineages in *Drosophila* derive from a common ancestral canonical lineage. A detailed analysis of the successive cell divisions in peripheral lineages (see below) reveals that they also share many molecular and cell biological properties, which strengthens their probable common evolutionary origin. Only two presently described sensory lineages are not fully comparable to the canonical lineage, the olfactory lineage and the dbd lineage. However, their molecular and cell biological properties lead us to argue that they may represent early evolutionary steps in the assembly of the canonical lineage. Thus, basic biological properties of present-day cell lineages may provide clues as to infer how this stereotyped pattern of cell divisions might have been assembled during evolution.

Control of fate asymmetry by spindles and crescents: pI- and pIIb-type divisions

As mentioned earlier, cell–cell signalling through the Delta–Notch pathway ensures that the daughter cells of every division in PNS lineages adopt distinct fates. Although both daughters are capable of sending and receiving signals through this pathway, the direction of signaling is usually made largely unidirectional by the unequal segregation of cell fate determinants into one of the daughter cells. Key determinants are localized to "crescents" on one side of the cell cortex before mitosis, and this is coordinated with the axis of division so that protein crescents are subsequently inherited by only one of the two daughters. In the past half-decade, a multitude of crescent-forming proteins and RNAs have been identified, along with much of the cell biological machinery that mediates their localization. One of the most studied determinants is Numb, which localizes asymmetrically during divisions of embryonic CNS neuroblasts and in all PNS lineages examined, including the microchaete lineage, embryonic campaniform lineage, and md-solo lineage (Chia and Yang, 2002; Rhyu et al., 1994). Numb appears to be one of the most proximate crescent-forming proteins in controlling N activity. It seems to negatively regulate N together with three other proteins, the endocytic protein α-Adaptin, the vesicle targeting protein Lethal Giant Larvae and the membrane protein Sanpodo (Berdnik et al., 2002; Justice et al., 2003; O'Connor-Giles and Skeath, 2003). However, its mechanism of action is still unknown.

Because determinant localization must be coordinated with orientation of the mitotic spindle, the orientations of cell divisions within sensory lineages are carefully controlled. This is manifest in successive and stereotyped remodeling of cell polarity within the lineage. This has been particularly noticed for the microchaete and embryonic campaniform lineages (Gho and Schweisguth, 1998; Gho et al., 1999; Orgogozo et al., 2001; Roegiers et al., 2001). In both lineages, the pI cell first acquires a characteristic spindle orientation. This distinguishes it from other epithelial cells, which divide with randomly oriented axes of division, although both types of cells divide within the epithelial plane. pIIb cell polarity is then remodeled into an apical-basal polarity and it divides perpendicularly to the plane of the epithelium. Later, the pIIa and pIIIb cell divide with an orientation similar to their mother cells, pI and pIIb, respectively. Similarly, in the md-solo lineage, pI cell division occurs within the plane of the epithelium, whereas the pIIb division occurs roughly perpendicular to the plane of the epithelium (Orgogozo et al., 2001). Thus, successive changes in cell polarity may be consistently related to the canonical lineage.

Neuroblasts resemble pIIb/pIIIb in that they also divide perpendicularly to the epithelial plane to generate daughter cells with distinct fates. Neuroblasts use the pre-existing apical–basal polarity of the epithelium to not only orient their axis of division, but localize determinants as well (reviewed by Chia and Yang, 2002). Key amongst these is Inscuteable (Insc), and the introduction of ectopic Insc in the normally Insc-negative epidermal cells is sufficient to reorient their division axis to be perpendicular to the epithelial plane (Kraut et al., 1996).

All proteins that localize asymmetrically in dividing neuroblasts (including Numb, Partner of Numb, Prospero, Miranda, Insc, Bazooka, and Partner of Inscuteable) have thus far been found in the same distinctive locations in dividing pIIb cells (Gho et al., 1999; Le Borgne et al., 2002; Orgogozo et al., 2001; Reddy and Rodrigues, 1999a; Roegiers et al., 2001). Additional mechanistic links between pIIb and neuroblast division include their dependence on the Insc/apical complex machinery and a strong asymmetry between the sizes of the daughter cells and their spindles, with the basal cell being smaller in both respects (Gho et al.,

1999; Reddy and Rodrigues, 1999a; Roegiers et al., 2001). Thus, pIIb cells and neuroblasts share many characteristics.

Because pI and pIIa cells divide within the epithelium plane, they must use other cues to orient their divisions. Accordingly, Insc does not accumulate in pI and pIIa cells and components of the apical complex are separated in microchaete pI cells: Bazooka/atypical protein kinase C (and presumably Par-6) are found posteriorly while Pins/Gαi are found anteriorly (Bellaïche et al., 2001b). In the case of microchaetes, their characteristic antero-posterior orientation of the division is controlled by components of the planar polarity pathway, most notably by Frizzled (Fz), a seven-transmembrane receptor of the Wnt family (Gho and Schweisguth, 1998). In *fz* mutants, Partner of Inscuteable/Gαi/Numb and Bazooka continue to form cortical crescents that localize to opposite poles but they no longer align along the antero-posterior axis (Bellaïche et al., 2001a,b; Roegiers et al., 2001).

To summarize, two types of divisions within the lineage are detected: neuroblast-like divisions that are perpendicular to the epithelial plane (pIIb and pIIIb) and divisions that are oriented but lie within the plane of the epithelium (pI and pIIa). These data raise the interesting possibility that the portion of peripheral sense organ lineages that generates internal cells (pIIb branch) may be ancestrally related to a neuroblast-like lineage. By contrast, the evolutionary origin of the asymmetric cell divisions occurring within the epithelial plane (pI and pIIa) remained particularly obscure, until a recent study of olfactory sensilla development.

Joining the club again: from cell recruitment to asymmetric cell division?

Clonal analysis demonstrated that cells of individual olfactory sensilla (Figs. 3J–L) are of mixed lineage (Reddy et al., 1997). During development, an identified olfactory precursor cell becomes first associated with several cells that together comprise a presensillum cluster in the absence of cell divisions. Then, cells of the presensillum cluster divide to give rise to differentiated cells of an olfactory sensillum (Ray and Rodrigues, 1995; Reddy et al., 1997).

Recent detailed analysis of presensillum cluster cell lineages in olfactory sensilla (Fig. 3L) have led to the rather unexpected conclusion that the olfactory precursor cells of a presensillum cluster are pIIa- and pIIb-like cells that execute lineages that are identical to those in the canonical PNS lineage (Fig. 4H) (Sen et al., 2003). Although not lineally related, the pIIa- and pIIb-like cells present strinking molecular and developmental similarities with their apparent counterparts in the canonical lineage. For example, Prospero regulates lineage identity and accumulates in pIIb-like cells and not in pIIa-like cells, as seen for microchaete and embryonic campaniform lineages. As in the canonical lineage, pIIa undergoes a single division to generate a socket and a shaft cell while pIIb undergoes two divisions to produce a neuron, a sheath cell, and a glial cell that undergoes apoptosis in some lineages (Figs. 4A, H) (Sen et al., 2003, 2004). A third cell was also detected in the presensillum cluster, pIIc, which was proposed to divide to generate two additional neurons of the olfactory sensilla. Its possible counterpart in the canonical lineage remains unclear.

This newly described lineage suggests an evolutionary model in which a neuroblast-related, pIIb-type cell lineage was present before the canonical sensory lineage. In this view, the hypothetical pIIb-derived sensillum may have recruited additional planarly dividing, pIIa-like, support cells from the neighboring epithelium. It is tempting to imagine that this involved Spitz/EGFR signaling, given its general usage in sensory organ cell recruitment. Subsequently, the recruitment of a pIIa-like cell by a pIIb-like cell may have been transformed into an asymmetric cell division that produces pIIa and pIIb cells related by lineage. According to this particular evolutionary scenario, the olfactory lineage may represent either an ancestral situation before the canonical lineage, or a reverse evolution back to the ancestral state. Two pieces of data will be necessary to substantiate this idea. First, it remains to be seen if presensillum cluster recruitment in the olfactory lineage is indeed mediated by EGFR signaling. Second, it will be necessary to assess whether the first olfactory precursor cell to appear is a pIIa- or a pIIb-type cell. Unfortunately, satisfactory markers do not yet exist to answer this second question; however, according to our proposed model, we would predict it to be a pIIb-like cell.

Numb reinforces an existing lineage asymmetry?

In all sensory organs examined to date [microchaetes, (Hartenstein and Posakony, 1990); wing campaniform organs, (Van De Bor and Giangrande, 2001); embryonic external sensory organs, (Hartenstein and Campos-Ortega, 1986; Vervoort et al., 1997)], reduction of Notch signaling transforms pIIa cells into pIIb cells, resulting in additional inner sensory cells and fewer outer cells. Consistent with this, Notch signaling is activated and required in the pIIa cell, whereas it is inhibited in its sibling pIIb cell following Numb asymmetric segregation into the pIIb cell [microchaete lineage, (Gho et al., 1999); embryonic campaniform lineage, (Orgogozo et al., 2001); md-solo lineage, (Orgogozo et al., 2002)].

Interestingly, in the olfactory presensillum cluster, the binary choice between pIIa and pIIb cell fates is also regulated by Notch. As seen with other sensory organs, inner olfactory cells develop at the expense of the outer cells when Notch signaling is reduced (Sen et al., 2003). Because these cells are not clonally related, this appears to be the only case so far of a N-regulated lineage cell fate choice that is not also regulated by asymmetric inheritance of Numb during mitosis. In agreement with the olfactory lineage bearing more ancestral characters than the canonical lineage, we suggest that usage of Numb to control the binary pIIa/pIIb fate decision may have come after a symmetry-breaking mechanism mediated by Notch alone. In a simple hypothetical

lineage comprising a single Notch-regulated cell division, stable activation of the Notch pathway in only one daughter might proceed via initially bidirectional signaling that stochastically resolves into unidirectional signaling to generates distinct daughter fates. This type of mechanism has been well characterized for the AC/VU cell fate decision during nematode vulval development. In this setting, N signaling between the non-lineally related cells Z1.ppp and Z4.aaa results in the random, but consistent, adoption of one cell as AC and other cell as VU (Greenwald, 1998).

Later, Numb may have been used to make signaling biased and thus increase inevitability of outcome. This is especially important during sensory organ development, where it is imperative that each of a small number of constituent cells adopts the correct fate. This scenario would also explain why *Notch* has such a general role in developmental cell fate choices, whereas *numb* has very specific roles, mostly in cell lineages.

Overall consideration of Notch-regulated cell fate decisions in mammals and flies, shows that Notch typically determines non-neuronal cell fates. This applies throughout the canonical lineage where Notch promotes the pIIa, pIIb, and sheath cell fates, versus the pIIb, md neuron, and sensory neuron fates, respectively. All of these observations are consistent with an ancestral function of Notch in diverting presumptive neuronal cells toward accessory fates within peripheral sensory lineages.

Interestingly, the *Drosophila* embryonic dbd lineage appears as a possible early step in the assembly of the canonical lineage. It consists of a single N-regulated division of a precursor cell to generate the multidendritic neuron dbd and an associated glial cell. In agreement with Notch inhibiting neural cell fates, Numb is inherited by the neuron and N is activated in the glial cell (Bodmer et al., 1989; Brewster and Bodmer, 1995; Umesono et al., 2002).

Aspects of the canonical lineage in other Metazoa

Very primitive sensory organs may have been composed solely of specialized neurons that directly sensed external stimuli. Sense organs of this sort are found in cnidarians, flatworms, nemertians, rotifera, annelids, and siponculids. In these species, most types of sensilla are in direct contact with the environment and are often associated with ciliated dendritic processes or a pit-like structure that allows exposure to the outside (reviewed by Bullock and Horridge, 1965; Wright, 1992). The evolution of additional non-neuronal support cells would have increased the sensitivity and sensing capabilities of sensory organs. For example, although some mechanoreceptors are embedded in the plane of the body wall, many others are associated with a rigid and lengthy bristle, which allows detection of physical stimuli at some distance from the body wall. In addition, support cells may have evolved in parallel with the development of the increasingly impenetrable cuticle or exoskeleton secreted by many higher invertebrates.

It is perhaps only a matter of philosophy to ask whether it is "better" to generate such accessory cells using a lineage or recruitment strategy. However, in the present-day fly, the collected analysis leads us to propose that the blueprints for the great majority of peripheral sensory organs are founded upon the same canonical lineage. We may never know whether the reason for the success of the canonical lineage is more an accident of history or if it reflects a genuine superiority in this strategy amongst other possibilities. However, we speculate that its incorporation of multiple layers of regulation that ensure maximal cell-type diversity and consolidation of the entire lineage into a single pI cell made it more "reliable" and stable as a platform for further evolutionary tinkering to generate sensory organ diversity, compared to a strategy in which portions of the lineage must be recruited.

Altogether then, our analysis leads us to propose that the canonical sensory lineage has been constructed during evolution through successive addition of a few developmental processes. These include Notch signaling to generate asymmetric cell divisions, asymmetric segregation of Numb during mitosis, variation in cell division number, cell recruitment via EGFR signaling, neuroblast-like cell division perpendicular to the epithelium and planar asymmetric cell division. It is important to note that these processes are not exclusive to sensory development. For instance, development of other nonneural *Drosophila* organs, such as muscle cells (Baylies et al., 1998), distal tip cells of Malpighian tubules (Hoch et al., 1994; Wan et al., 2000), and likely cardiac cells (Ward and Skeath, 2000), is initiated by proneural genes and proceeds via stereotyped Notch- and Numb-regulated cell lineages. It is thus conceivable that the canonical sensory lineage was constructed by developmental mechanisms previously used in nonsensory tissues.

Morphologically similar types of peripheral sensory organs are evident in other insects. While it is tempting to suppose that these also derive from canonical lineages, we must exercise caution in making this connection in the absence of detailed lineage study, as advised by the microchaete lineage history (see above). For example, socket and scale cells of Lepidopteran wing scales appear to be analogous to the socket and shaft cells of fly bristle organs, respectively. This thinking is informed by their physical resemblance, common polyploid status, and the sibling relationship of the cell pairs. Nevertheless, division of pI cells for Lepidopteran wing scales has not been found to be planar, but in fact perpendicular to the epithelial plane (Stossberg, 1938). If true, this would be a fundamental departure from the canonical *Drosophila* peripheral lineage. Similar lability in developmental strategy underlying nematode vulval development is hidden by the similar end-product in different species (Gibson, 2001).

Nevertheless, at least two studies clearly indicate the presence of the canonical lineage in other insects. First, in

the moth *Manduca sexta*, BrdU incorporation studies indicate that an md neuron is associated by lineage with the four-cell larval bristle and originates from a pIIb-like cell that executes a lineage identical to the one in the canonical lineage (Grueber and Truman, 1999). Second, a lineage described in the milkweed bug *Oncopeltus fasciatus* some 38 years ago corresponds exactly to the canonical lineage. SOPs of mechanosensory organs in this insect were observed to divide within the plane of the epithelium. Then, one daughter cell divides perpendicularly to the plane of the epithelium (pIIb) before its sister cell divides within the plane of the epithelium and generates two outer cells (pIIa). Two outer and three inner cells are produced, with one of the inner cells undergoing nuclear breakdown (a possible sign of apoptosis), thus leaving four surviving cells in the mature organ (Lawrence, 1966). This suggests that in addition to the canonical lineage, the microchaete lineage itself may have been present in their common ancestor.

Analyses of neural lineages in other species have been most rigorously performed in *C. elegans*, for which the lineage of the entire organism has been described (Sulston and Horvitz, 1977; Sulston et al., 1983). Of the 10 different types of sensilla, 5 are composed of cells that originate from different lineage branches and subsequently cluster together. Of the remaining 5 that contain at least some cells related by lineage, 4 can be plausibly related to the canonical *Drosophila* lineage, with the male post-cloacal sensory lineage being most similar. As in *Drosophila*, the *atonal* ortholog is required to specify neuronal and sensory cell fates in *C. elegans* (Portman and Emmons, 2000; Zhao and Emmons, 1995). The operation of the core Notch pathway has also been conserved in *C. elegans* (Greenwald, 1998), but it has not been characterized with respect to these particular neural lineages. In addition, although Numb and Prospero orthologs are present in nematodes, neither has been studied genetically (Ruvkun and Hobert, 1998). Thus, while the general similarity between these nematode lineages and the canonical insect lineage is tantalizing, their ancestral relationship cannot be adequately assessed at present.

A similarly incomplete situation applies to vertebrates. Conservation of the genetic machinery is compelling: proneural bHLH-encoding genes, the Notch pathway, and Numb are used in a rather similar fashion to initiate neurogenesis and regulate asymmetric neural cell divisions (Bertrand et al., 2002; Cayouette and Raff, 2002). Unfortunately, events within sensory lineages are not easily amenable to in vivo temporal analysis with single-cell resolution as in flies and worms. Nonetheless, vertebrate CNS development may use defined sublineages (Qian et al., 1998) and several parallels are observed between the genetic developmental pathways and physiology of the mammalian inner ear and *Drosophila* sensory organs. This suggest that the mammalian inner ear may be potentially evolutionarily related to *Drosophila* sensory organs and share common aspects of lineage (Adam et al., 1998; Eddison et al., 2000).

In conclusion, this comparative developmental analysis strongly suggests that the canonical lineage predates the separation of Holometabola (fly) from Hemipteroid (milkweed bug) (which is estimated to be >350 million years old). Whether the canonical lineage is much older remains to be investigated.

Concluding remarks

The history of our understanding of the mechanosensory lineage and other *Drosophila* sensory lineages embodies two optimistic philosophical perspectives of scientific pursuit. The first is that novelty continues to arise even in extremely well-studied model systems. This underscores the hopeful proposition that things in nature are almost always more complicated than we expect, and suggests that in spite of what we know, there is still much left to discover. In fact, despite all that has been learned thus far, the book is not yet closed with regard to understanding sensory lineages. For example, the molecular mechanisms regulating the temporal sequence of the developmental events hard-wired by SOPs are totally unknown. Sequential generation of different cell types through successive mitosis during development seems to be a widespread developmental mechanism in Metazoa (Salazar-Ciudad et al., 2003) and very little is known about this process. The wide variety of *Drosophila* sensory lineages described here provide ideal settings in which to investigate this matter.

A second precept is that despite life's complications, careful studies can reveal connections between disparate systems. The relationships between diverse peripheral sense organ lineages, which seem so clear and obvious in retrospect, depended upon multiple revisions and re-interpretations of existing data. Nevertheless, connections were successfully made following years of meticulous analyses. This gives hope that we may be able to eventually understand something as amorphous and overwhelming as the question of how life is assembled.

Acknowledgments

Many colleagues engaged in enlightening discussions, shared unpublished observations, and provided useful critiques of this review, including Adina Bailey, Yohanns Bellaïche, Chris Doe, Marie-Anne Felix, Michel Gho, Angela Giangrande, Volker Hartenstein, Thomas Keil, Laurina Manning, Veronica Rodrigues, Fabrice Roegiers, Francois Schweisguth, Pat Simpson, and Michel Vervoort. Their input was invaluable and gratefully acknowledged. We also thank Wesley Grueber for the md neuron image (Fig. 3B) and Veronica Rodrigues for images of olfactory sensilla (Figs. 3J–L). E.C.L. recognizes the gracious support of Gerald Rubin and the Damon Runyon Cancer Research Foundation, DRG 1632. V.O.'s work was supported by

grants to Francois Schweisguth from the Centre National de la Recherche Scientifique and Association pour la Recherche sur le Cancer (ARC4512).

References

Adam, J., Myat, A., Le Roux, I., Eddison, M., Henrique, D., Ish-Horowicz, D., Lewis, J., 1998. Cell fate choices and the expression of Notch, Delta and Serrate homologues in the chick inner ear: parallels with *Drosophila* sense-organ development. Development 125, 4645–4654.

Awasaki, T., Kimura, K., 1997. *pox-neuro* is required for development of chemosensory bristles in *Drosophila*. J. Neurobiol. 32, 707–721.

Awasaki, T., Kimura, K., 2001. Multiple function of poxn gene in larval PNS development and in adult appendage formation of *Drosophila*. Dev. Genes Evol. 211, 20–29.

Bate, M., 1978. Development of sensory systems in Arthropods. In: Jakobson, M. (Ed.), Handbook of Sensory Physiology. Springer, Berlin, pp. 1–53.

Baylies, M.K., Bate, M., Ruiz Gomez, M., 1998. Myogenesis: a view from *Drosophila*. Cell 93, 921–927.

Bellaïche, Y., Gho, M., Kaltschmidt, J.A., Brand, A.H., Schweisguth, F., 2001a. Frizzled regulates localization of cell-fate determinants and mitotic spindle rotation during asymmetric cell division. Nat. Cell Biol. 3, 50–57.

Bellaïche, Y., Radovic, A., Woods, D.F., Hough, C.D., Parmentier, M.L., O'Kane, C.J., Bryant, P.J., Schweisguth, F., 2001b. The partner of inscuteable/discs-large complex is required to establish planar polarity during asymmetric cell division in *Drosophila*. Cell 106, 355–366.

Berdnik, D., Torok, T., Gonzalez-Gaitan, M., Knoblich, J.A., 2002. The endocytic protein alpha-Adaptin is required for numb-mediated asymmetric cell division in *Drosophila*. Dev. Cell 3, 221–231.

Bergmann, A., Tugentman, M., Shilo, B.Z., Steller, H., 2002. Regulation of cell number by MAPK-dependent control of apoptosis: a mechanism for trophic survival signaling. Dev. Cell 2, 159–170.

Berlese, A., 1909. Sistema periferico sensoriale. Gli Insetti 1, 601–699.

Bertrand, N., Castro, D., Guillemot, F., 2002. Proneural genes and the specification of neural cell types. Nat. Rev., Neurosci. 3, 517–530.

Blochlinger, K., Bodmer, R., Jan, L.Y., Jan, Y.N., 1990. Patterns of expression of Cut, a protein required for external sensory organ development, in wild-type and *cut* mutant *Drosophila* embryos. Genes Dev. 4, 1322–1331.

Blochlinger, K., Jan, L.Y., Jan, Y.N., 1991. Transformation of sensory organ identity by ectopic expression of Cut in *Drosophila*. Genes Dev. 5, 1124–1135.

Bodmer, R., Jan, Y.N., 1987. Morphological differentiation of the embryonic peripheral neurons in *Drosophila*. Roux's Arch. Dev. Biol. 196, 69–77.

Bodmer, R., Barbel, S., Sheperd, S., Jack, J.W., Jan, L.Y., Jan, Y.N., 1987. Transformation of sensory organs by mutations of the *cut* locus of *D. melanogaster*. Cell 51, 293–307.

Bodmer, R., Carretto, R., Jan, Y.N., 1989. Neurogenesis of the peripheral nervous system in *Drosophila* embryos: DNA replication patterns and cell lineages. Neuron 3, 21–32.

Boulianne, G.L., de la Concha, A., Campos-Ortega, J.A., Jan, L.Y., Jan, Y.N., 1991. The *Drosophila* neurogenic gene *neuralized* encodes a novel protein and is expressed in precursors of larval and adult neurons. EMBO J. 10, 2975–2983.

Brewster, R., Bodmer, R., 1995. Origin and specification of type II sensory neurons in *Drosophila*. Development 121, 2923–2936.

Bullock, T.H., Horridge, G.A., 1965. Structure and Function in the Nervous Systems of Invertebrates. Freeman, San Francisco.

Cagan, R.L., Ready, D.F., 1989. The emergence of order in the *Drosophila* pupal retina. Dev. Biol. 136, 346–362.

Campbell, G., Goring, H., Lin, T., Spana, E., Andersson, S., Doe, C.Q., Tomlinson, A., 1994. RK2, a glial-specific homeodomain protein required for embryonic nerve cord condensation and viability in *Drosophila*. Development 120, 2957–2966.

Campos-Ortega, J.A., Hartenstein, V., 1985. The embryonic development of *Drosophila melanogaster*. Springer-Verlag, Berlin.

Cayouette, M., Raff, M., 2002. Asymmetric segregation of Numb: a mechanism for neural specification from *Drosophila* to mammals. Nat. Neurosci. 5, 1265–1269.

Chia, W., Yang, X., 2002. Asymmetric division of *Drosophila* neural progenitors. Curr. Opin. Genet. Dev. 12, 459–464.

Dambly-Chaudiere, C., Jamet, E., Burri, M., Bopp, D., Basler, K., Hafen, E., Dumont, N., Spielmann, P., Ghysen, A., Noll, M., 1992. The paired box gene pox neuro: a determinant of poly-innervated sense organs in *Drosophila*. Cell 69, 159–172.

del Alamo, D., Terriente, J., Diaz-Benjumea, F.J., 2002. Spitz/EGFr signalling via the Ras/MAPK pathway mediates the induction of bract cells in *Drosophila* legs. Development 129, 1975–1982.

Doe, C.Q., Chu-LaGraff, Q., Wright, D.M., Scott, M.P., 1991. The *prospero* gene specifies cell fates in the *Drosophila* central nervous system. Cell 65, 451–464.

Eberl, D.F., Hardy, R.W., Kernan, M.J., 2000. Genetically similar transduction mechanisms for touch and hearing in *Drosophila*. J. Neurosci. 20, 5981–5988.

Eddison, M., Le Roux, I., Lewis, J., 2000. Notch signaling in the development of the inner ear: lessons from *Drosophila*. Proc. Natl. Acad. Sci. U. S. A. 97, 11692–11699.

Elstob, P.R., Brodu, V., Gould, A.P., 2001. Spalt-dependent switching between two cell fates that are induced by the *Drosophila* EGF receptor. Development 128, 723–732.

Fichelson, P., Gho, M., 2003. The glial cell undergoes apoptosis in the microchaete lineage of *Drosophila*. Development 130, 123–133.

Franzl, W., 1941. Die Cytogenese der bipolaren Sinneszellen bei der Larve von Dytiscus sp. Z. Zellforsch. 31, 54–59.

Freeman, M., 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell 87, 651–660.

Galant, R., Skeath, J.B., Paddock, S., Lewis, D.L., Carroll, S.B., 1998. Expression pattern of a butterfly achaete-scute homolog reveals the homology of butterfly wing scales and insect sensory bristles. Curr. Biol. 8, 807–813.

Gho, M., Schweisguth, F., 1998. Frizzled signalling controls orientation of asymmetric sense organ precursor cell divisions in *Drosophila*. Nature 393, 178–181.

Gho, M., Bellaïche, Y., Schweisguth, F., 1999. Revisiting the *Drosophila* microchaete lineage: a novel intrinsically asymmetric cell division generates a glial cell. Development 126, 3573–3584.

Ghysen, A., O'Kane, C., 1989. Neural enhancer-like elements as specific cell markers in *Drosophila*. Development 105, 35–52.

Ghysen, A., Dambly-Chaudiere, C., Aceves, E., Jan, L.Y., Jan, Y.N., 1986. Sensory neurons and peripheral pathways in *Drosophila* embryos. Roux's Arch. Dev. Biol. 195, 281–289.

Gibson, G., 2001. Developmental evolution: the unbearable likeness of beings. Curr. Biol. 11, R345–R348.

Gnatzy, W., 1976. The ultrastructure of the thread-hairs on the cerci of the cockroach *Periplaneta americana* L.: the intermoult phase. J. Ultrastruct. Res. 54, 124–134.

Gnatzy, W., Schmidt, K., 1971. Die Feinstruktur der Sinneshaare auf den Cerci von *Gryllus bimaculatus* Deg. (Saltatoria, Gryllidae): I. Faden- und Keulenhaare. Z. Zellforsch. 122, 190–209.

Greenwald, I., 1998. LIN-12/Notch signaling: lessons from worms and flies. Genes Dev. 12, 1751–1762.

Grueber, W.B., Truman, J.W., 1999. Development and organization of a nitric-oxide-sensitive peripheral neural plexus in larvae of the moth, *Manduca sexta*. J. Comp. Neurol. 404, 127–141.

Grueber, W.B., Jan, L.Y., Jan, Y.N., 2002. Tiling of the *Drosophila* epidermis by multidendritic sensory neurons. Development 129, 2867–2878.

Haffer, O., 1921. Bau und Funktion der Sternwarzen von *Saturnia pyri*.

Schiff., und die Haarentwicklung der Saturniden-Raupen. Arch. Naturgesch., 110–166.
Halter, D.A., Urban, J., Rickert, C., Ner, S.S., Ito, K., Travers, A.A., Technau, G.M., 1995. The homeobox gene repo is required for the differentiation and maintenance of glia function in the embryonic nervous system of *Drosophila melanogaster*. Development 121, 317–332.
Hartenstein, V., 1988. Development of *Drosophila* larval sensory organs: spatiotemporal pattern of sensory neurones, peripheral axonal pathways and sensilla differentiation. Development 102, 869–886.
Hartenstein, V., Campos-Ortega, J.A., 1986. The peripheral nervous system of mutants of early neurogenesis in *Drosophila melanogaster*. Roux's Arch. Dev. Biol. 195, 210–221.
Hartenstein, V., Jan, Y.N., 1992. Studying *Drosophila* embryogenesis with P-*lacZ* enhancer trap lines. Roux's Arch. Dev. Biol. 201, 194–220.
Hartenstein, V., Posakony, J.W., 1989. Development of adult sensilla on the wing and notum of *Drosophila melanogaster*. Development 107, 389–405.
Hartenstein, V., Posakony, J.W., 1990. A dual function of the *Notch* gene in *Drosophila* sensillum development. Dev. Biol. 142, 13–30.
Held Jr., L.I., 2002. Bristles induce bracts via the EGFR pathway on *Drosophila* legs. Mech. Dev. 117, 225–234.
Hertweck, H., 1931. Anatomie und Variabilität des nervensystems und der Sinnesorgane von *Drosophila melanogaster* (Meigen). Z. Wiss. Zool. 139, 559–663.
Hoch, M., Broadie, K., Jackle, H., Skaer, H., 1994. Sequential fates in a single cell are established by the neurogenic cascade in the Malpighian tubules of *Drosophila*. Development 120, 3439–3450.
Huang, F., Dambly-Chaudière, C., Ghysen, A., 1991. The emergence of sense organs in the wing disc of *Drosophila*. Development 111, 1087–1095.
Jan, Y.N., Jan, L.Y., 1993. The peripheral nervous system. In: Bate, M., Martinez-Arias, A. (Eds.), The Development of *Drosophila melanogaster*, vol. I. Cold Spring Harbor Laboratory Press, New York, pp. 1207–1244.
Jarman, A.P., Ahmed, I., 1998. The specificity of proneural genes in determining *Drosophila* sense organ identity. Mech. Dev. 76, 117–125.
Jones, B.W., 2001. Glial cell development in the *Drosophila* embryo. BioEssays 23, 877–887.
Jones, B.W., Fetter, R.D., Tear, G., Goodman, C.S., 1995. Glial cells missing: a genetic switch that controls glial versus neuronal fate. Cell 82, 1013–1023.
Justice, N., Roegiers, F., Jan, L.Y., Jan, Y.N., 2003. Lethal giant larvae acts together with numb in notch inhibition and cell. Curr. Biol. 13, 778–783.
Keil, T., 1978. Die Makrochaeten auf dem Thorax von Calliphora vicina Robineau-Desvoidy (Calliphoridae, Diptera). Feinstruktur und Morphogenese eines epidermalen Insekten-Mechanoreceptors. Zoomorphologie 90, 151–180.
Kraut, R., Chia, W., Jan, L.Y., Jan, Y.N., Knoblich, J.A., 1996. Role of *inscuteable* in orienting asymmetric cell divisions in *Drosophila*. Nature 383, 50–55.
Lawrence, P.A., 1966. Development and determination of hairs and bristles in the milkweed bug, *Oncopeltus fasciatus*. J. Cell Sci. 1, 475–498.
Le Borgne, R., Bellaiche, Y., Schweisguth, F., 2002. *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. Curr. Biol. 12, 95–104.
Lees, A.D., Waddington, C.H., 1942. The development of the bristles in normal and some mutant types of *Drosophila melanogaster*. Proc. R. Soc., Ser. B 131, 87–110.
Manning, L., Doe, C.Q., 1999. Prospero distinguishes sibling cell fate without asymmetric localization in the *Drosophila* adult external sense organ lineage. Development 126, 2063–2071.
Matthews, K.A., Miller, D.F., Kaufman, T.C., 1990. Functional implications of the unusual spatial distribution of a minor alpha-tubulin isotype in *Drosophila*: a common thread among chordotonal ligaments, developing muscle, and testis cyst cells. Dev. Biol. 137, 171–183.
Mayer, U., Nusslein, V.C., 1988. A group of genes required for pattern formation in the ventral ectoderm of the *Drosophila* embryo. Genes Dev. 2, 1496–1511.
Merritt, D.J., 1997. Transformation of external sensilla to chordotonal sensilla in the cut mutant of *Drosophila* assessed by single-cell marking in the embryo and larva. Microsc. Res. Tech. 39, 492–505.
Merritt, D.J., Hawken, A., Whitington, P.M., 1993. The role of the cut gene in the specification of central projections by sensory axons in *Drosophila*. Neuron 10, 741–752.
Moulins, M., 1976. Ultrastructure of chordotonal organs. In: Mill, P. (Ed.), Structure and Function of Proprioceptors in the Invertebrates. Chapman & Hall, London, pp. 387–426.
Murray, M.A., Schubiger, M., Palka, J., 1984. Neuron differentiation and axon growth in the developing wing of *Drosophila melanogaster*. Dev. Biol. 104, 259–273.
Nayak, S., Singh, S., 1983. Sensilla on the tarsal segments and mouthparts of adult *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Int. J. Insect Morphol. Embryol. 12, 273–291.
Noordermeer, J.N., Kopczynski, C.C., Fetter, R.D., Bland, K.S., Chen, W.Y., Goodman, C.S., 1998. Wrapper, a novel member of the Ig superfamily, is expressed by midline glia and is required for them to ensheath commissural axons in *Drosophila*. Neuron 21, 991–1001.
Nottebohm, E., Ramaekers, A., Dambly-Chaudiere, C., Ghysen, A., 1994a. The leg of *Drosophila* as a model system for the analysis of neuronal diversity. J. Physiol. (Paris) 88, 141–151.
Nottebohm, E., Usui, A., Therianos, S., Kimura, K., Dambly-Chaudiere, C., Ghysen, A., 1994b. The gene poxn controls different steps of the formation of chemosensory organs in *Drosophila*. Neuron 12, 25–34.
O'Connor-Giles, K.M., Skeath, J.B., 2003. Numb inhibits membrane localization of Sanpodo, a four-pass transmembrane. Dev. Cell 5, 231–243.
Okabe, M., Okano, H., 1997. Two-step induction of chordotonal organ precursors in *Drosophila* embryogenesis. Development 124, 1045–1053.
Okano, H., Hayashi, S., Tanimura, T., Sawamoto, K., Yoshikawa, S., Watanabe, J., Iwasaki, M., Hirose, S., Mikoshiba, K., Montell, C., 1992. Regulation of *Drosophila* neural development by a putative secreted protein. Differentiation 52, 1–11.
Orgogozo, V., Schweisguth, F., Bellaïche, Y., 2001. Lineage, cell polarity and inscuteable function in the peripheral nervous system of the *Drosophila* embryo. Development 128, 631–643.
Orgogozo, V., Schweisguth, F., Bellaiche, Y., 2002. Binary cell death decision regulated by unequal partitioning of Numb at mitosis. Development 129, 4677–4684.
Parks, A.L., Muskavitch, M.A., 1993. *Delta* function is required for bristle organ determination and morphogenesis in *Drosophila*. Dev. Biol. 157, 484–496.
Perry, M.M., 1968. Further studies on the development of the of *Drosophila melanogaster*: II. The interommatidial bristles. J. Morphol. 124, 249–262.
Peters, W., 1963. Die Sinnesorgane an des Labellen von *Calliphora erythrocephala* Mg. Diptera 55, 259–320.
Portman, D.S., Emmons, S.W., 2000. The basic helix–loop–helix transcription factors LIN-32 and HLH-2 function together in multiple steps of a *C. elegans* neuronal sublineage. Development 127, 5415–5426.
Posakony, J.W., 1994. Nature versus nurture: asymmetric cell divisions in *Drosophila* bristle development. Cell 76, 415–418.
Qian, X., Goderie, S.K., Shen, Q., Stern, J.H., Temple, S., 1998. Intrinsic programs of patterned cell lineages in isolated vertebrate CNS ventricular zone cells. Development 125, 3143–3152.
Raff, M.C., Barres, B.A., Burne, J.F., Coles, H.S., Ishizaki, Y., Jacobson, M.D., 1993. Programmed cell death and the control of cell survival: lessons from the nervous system. Science 262, 695–700.
Ray, K., Rodrigues, V., 1995. Cellular events during development of the olfactory sense organs in *Drosophila melanogaster*. Dev. Biol. 167, 426–438.
Ray, K., Hartenstein, V., Rodrigues, V., 1993. Development of the taste

bristles on the labellum of *Drosophila melanogaster*. Dev. Biol. 155, 26–37.

Reddy, G.V., Rodrigues, V., 1999a. A glial cell arises from an additional division within the mechanosensory lineage during development of the microchaete on the *Drosophila* notum. Development 126, 4617–4622.

Reddy, G.V., Rodrigues, V., 1999b. Sibling cell fate in the *Drosophila* adult external sense organ lineage is specified by prospero function, which is regulated by Numb and Notch. Development 126, 2083–2092.

Reddy, G.V., Gupta, B., Ray, K., Rodrigues, V., 1997. Development of the *Drosophila* olfactory sense organs utilizes cell–cell interactions as well as lineage. Development 124, 703–712.

Rhyu, M.S., Jan, L.Y., Jan, Y.N., 1994. Asymmetric distribution of numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. Cell 76, 477–491.

Ribbert, D., 1967. The polytene chromosomes of bristle-forming-cells of *Calliphora erythrocephala* with special consideration of sex-linked structural heterozygosity and puff patterns during metamorphosis. Chromosoma 21, 296–344.

Robertson, C.W., 1936. The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes. J. Morph. 59, 351–391.

Roegiers, F., Younger-Shepherd, S., Jan, L.Y., Jan, Y.N., 2001. Two types of asymmetric divisions in the *Drosophila* sensory organ precursor cell lineage. Nat. Cell Biol. 3, 58–67.

Rusten, T.E., Cantera, R., Urban, J., Technau, G., Kafatos, F.C., Barrio, R., 2001. Spalt modifies EGFR-mediated induction of chordotonal precursors in the embryonic PNS of *Drosophila* promoting the development of oenocytes. Development 128, 711–722.

Ruvkun, G., Hobert, O., 1998. The taxonomy of developmental control in *Caenorhabditis elegans*. Science 282, 2033–2041.

Salazar-Ciudad, I., Jernvall, J., Newman, S.A., 2003. Mechanisms of pattern formation in development and evolution. Development 130, 2027–2037.

Schweisguth, F., Gho, M., Lecourtois, M., 1996. Control of cell fate choices by lateral signaling in the adult peripheral nervous system of *Drosophila melanogaster*. Dev. Genet. 18, 28–39.

Sen, A., Reddy, G.V., Rodrigues, V., 2003. Combinatorial expression of Prospero, Seven-up and Elav identifies progenitor cell types during sense-organ differentiation in the *Drosophila* antenna. Dev. Biol., 253.

Sen, A., Kuruvilla, D., Pinto, L., Sarin, A., Rodrigues, V., 2004. Programmed cell death and context dependent activation of the EGF pathway regulate gliogenesis in the *Drosophila* olfactory system. Mech. Dev. 121, 65–78.

Sonnenfeld, M.J., Jacobs, J.R., 1995. Apoptosis of the midline glia during *Drosophila* embryogenesis: a correlation with axon contact. Development 121, 569–578.

Stern, C., 1938. The innervation of setae in *Drosophila*. Genetics 23, 172–173.

Stossberg, M., 1938. Die Zellvorgänge bei der Entwicklung der Flügelschuppen von *Ephestia kühniella*. Z. Morphol. Ökol. Tiere 34, 173–206.

Sulston, J.E., Horvitz, H.R., 1977. Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. 56, 110–156.

Sulston, J.E., Schierenberg, E., White, J., Thomson, J., 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. 100, 64–119.

Umesono, Y., Hiromi, Y., Hotta, Y., 2002. Context-dependent utilization of Notch activity in *Drosophila* glial determination. Development 129, 2391–2399.

Usui, K., Kimura, K., 1993. Sequential emergence of the evenly spaced microchaetes on the notum of *Drosophila*. Roux's Arch. Dev. Biol. 203, 151–158.

Vaessin, H., Grell, E., Wolff, E., Bier, E., Jan, L.Y., Jan, Y.N., 1991. prospero is expressed in neuronal precursors and encodes a nuclear protein that is involved in the control of axonal outgrowth in *Drosophila*. Cell 67, 941–953.

Van De Bor, V., Giangrande, A., 2001. Notch signaling represses the glial fate in fly PNS. Development 128, 1381–1390.

Van De Bor, V., Walther, R., Giangrande, A., 2000. Some fly sensory organs are gliogenic and require glide/gcm in a precursor that divides symmetrically and produces glial cells. Development 127, 3735–3743.

Vervoort, M., Zink, D., Pujol, N., Victoir, K., Dumont, N., Ghysen, A., Dambly-Chaudière, C., 1995. Genetic determinants of sense organ identity in *Drosophila*: regulatory interactions between cut and poxn. Development 121, 3111–3120.

Vervoort, M., Merritt, D.J., Ghysen, A., Dambly-Chaudiere, C., 1997. Genetic basis of the formation and identity of type I and type II neurons in *Drosophila* embryos. Development 124, 2819–2828.

Vogel, R., 1923. Zur Kenntnis des feineren Baues der Geruchsorgane der Wespen und Bienen. Z. Wiss. Zool. 120, 281–324.

Wan, S., Cato, A.M., Skaer, H., 2000. Multiple signalling pathways establish cell fate and cell number in *Drosophila* malpighian tubules. Dev. Biol. 217, 153–165.

Ward, E.J., Skeath, J.B., 2000. Characterization of a novel subset of cardiac cells and their progenitors in the *Drosophila* embryo. Development 127, 4959–4969.

White, K., Grether, M.E., Abrams, J.M., Young, L., Farrell, K., Steller, H., 1994. Genetic control of programmed cell death in *Drosophila*. Science 264, 677–683.

Wolff, T., Ready, D.F., 1991. Cell death in normal and rough eye mutants of *Drosophila*. Development 113, 825–839.

Wright, K.A., 1992. Peripheral sensilla of some lower invertebrates: the Platyhelminthes and Nematoda. Microsc. Res. Tech. 22, 285–297.

Xiong, W.C., Okano, H., Patel, N.H., Blendy, J.A., Montell, C., 1994. repo encodes a glial-specific homeo domain protein required in the *Drosophila* nervous system. Genes Dev. 8, 981–994.

Zawarzin, A., 1912. Histologische Studien über Insekten: II. Das sensible Nervensystem der *Aeschna* Larven. Z. Wiss. Zool. 100, 245–286.

Zhao, C., Emmons, S.W., 1995. A transcription factor controlling development of peripheral sense organs in *C. elegans*. Nature 373, 74–78.

Zhou, L., Schnitzler, A., Agapite, J., Schwartz, L.M., Steller, H., Nambu, J.R., 1997. Cooperative functions of the reaper and head involution defective genes in the programmed cell death of *Drosophila* central nervous system midline cells. Proc. Natl. Acad. Sci. U. S. A. 94, 5131–5136.

zur Lage, P., Jarman, A.P., 1999. Antagonism of EGFR and Notch signaling in the reiterative recruitment of *Drosophila* adult chordotonal sense organ precursors. Development 126, 3149–3157.

zur Lage, P., Jan, Y.N., Jarman, A.P., 1997. Requirement for EGF receptor signalling in neural recruitment during formation of *Drosophila* chordotonal sense organ clusters [see comments]. Curr. Biol. 7, 166–175.